Evaluation of Antimalarial Properties of *Ficus Platyphylla Del* Leaf Extract in Mice

ABSTRACT

Aim
This study was aim at investigating the effect of crude petroleum ether leaf extract of *Ficus platyphylla* Del on *Plasmodium berghei* infected mice.
Place and Duration of study
This research was carried out at the department of biochemistry, Federal university of technology minna, Niger state Nigeria in 2014.
Methodology
The crude plant extract of *F. platyphylla* was administered 72 hours at different doses post and pre infection for both the curative and prophylactic study respectively against residual infection. Mice were divided into 5 groups of 5 mice each, 3 of the groups where administered crude plants extract of *F. platyphaylla* at different doses (200, 400 and 600mg/kg body weight) while the other two serve as negative and positive control group and were administered 0.5ml and 50mg/kg body weight respectively.
Result
The extract at all doses produced significant ($P<0.05$) dose dependent chemo-suppressive activity with % inhibition of 38%, 61%, 74% and 81.8% for curative studies and 36.0%, 38.5%, 49.5% and 63.4% for prophylactic studies against the parasites at doses of 200mg/kgbw, 400mg/kgbw, 600mg/kgbw of the extract and 50mg/kgbw of Artesunate. All doses of the extract increased the survival time of the infected mice compared to the negative control group that was administered 0.5ml normal saline. The variation in the values of Packed Cell Volume (PCV) for treated group before and after extract administration was not significant at ($P<0.05$). The phytochemical screening of the plant extract showed the presence of tannin, saponin, flavonoids, terpenoids, steroids, anthroquinone and phenol.
Conclusion
The result of this study shows that *F. Platyphylla* leaf extract exhibited some antiplasmodial activity that could be exploited for safe, effective and affordable antimalaria regimen.

Keywords: Malaria, Ficus platyphylla, Plasmodium berghei, Anti- malarial, Suppressive.

INTRODUCTION

Despite efforts put in by government and non-governmental organization all over the world towards the eradication of malaria; the causative pathogen has thrived over the years, spreading far beyond their evolutionary origins in Africa and Southern Asia. Malaria still

remains a leading cause of death in most developing countries, especially in Africa. There are estimated to be 300-500 million clinical cases of malaria annually [1] and it is estimated to cause more than one million deaths annually, majority of which are children [2]. The parasite exhibits its activity by cleaving the erythrocytes of its host immediately after the release of the merozoites into the system. It achieves this with the aid of the protease enzyme whose major function is to catalyze the breakdown of other proteins by hydrolyzing their peptide bonds. Hence, clinical symptoms and signs of malaria occur shortly before or at the time of red blood cells lysis. The associated fever is caused by the release of merozoites, malaria pigment, parasites proteins and cellular debris. Chills or rigor followed by high fever are observed normally in a cyclical pattern [3].

Medicinal plants material remains an important source to combats serious disease in the world, being a store house to thousands of therapeutic phytochemicals. It has been instrumental in traditional medicines to treat different disease from time immemorial in various part of the world. In this regard, the first antimalarial was discovered by accident from the barks extracts of *Cinchona* (Rubiaceae) species.

Ficus platyphylla Del Holl (Moraceae) is a deciduous plant locally known as ‘Gamji’’ among the Hausa tribes in Nigeria, West Africa. It is widely distributed throughout the savannah region of West African Coast. The leaves and stem bark of the plant are used traditionally to treat malaria and anaemia [4]. Methanolic extracts of *F. platyphylla* barks have been previously shown to possess significant anti-inflammatory effects [5]. A study has also shown that the extract contains physoactive metabolites with potentials as an anti-epileptic agent [6].

It is in view of these, that this study is aimed at evaluating the antimalaria properties of crude leaf extract of *F. platyphylla* Del in mice.

MATERIALS AND METHOD

PLANT MATERIALS

Fresh leaves of the plants were collected from Lokoja, North Central Nigeria and were identified at the Department of Biological Science Ahmadu Bello University Zaria, Nigeria where a voucher specimen was deposited at the departmental herbarium.

RODENT PARASITE (*PLASMODIUM BERGHEI BERGHEI*)

The rodent parasite *Plasmodium berghei berghei* NK 65 used for the study was obtained from National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria and kept alive by continuous intra peritoneal passage in mice every four days at the Department of Biochemistry Federal University of Technology Minna, Nigeria [7].

ANIMALS

Healthy Swiss albino mice of both sex of about 6 weeks old weighing 20-25g each was used for the experiments. The animals were fed *ad libitum* with standard feed and had free access to water. They were also maintained under standard conditions of humidity, temperature and 12 hours light/darkness cycle. Experiment were conducted in strict compliance with internationally accepted principle for laboratory animal use and care as contained in the Canadian council on animal care guidelines and protocol review [8].

METHODS

PARASITE INOCULATION

The method described by Kabiru *et al.,* [9] was used for the inoculation of parasite into experimental animals. The inoculums consisted of $5x10^7$ of *P. berghei berghei* parasitized erythrocytes per ml. This was carried out by determining both the percentage parasitaemia and erythrocytes count of the donor mouse and diluting the blood with phosphate buffer saline in proportions indicated by both determinations.

PREPARATION OF CRUDE EXTRACTS

Fifty (50) grams of powdered leaves of *F. platyphylla* was weighed and macerated in 250ml of 100% petroleum ether, the extraction lasted for 2 hours, thereafter it was filtered hot using muslin cloth and solvents removed under reduced pressure using a water bath. Extract

obtained was transferred into a sterile universal bottle and kept in the refrigerator at 4°C until required for use [10].

PHYTOCHEMICAL SCREENING

To elucidate the secondary metabolites present, the crude leave extract was subjected to qualitative phytochemical screening using the methods of Odebiyi and Soforowa, [11].

RANE (CURATIVE TEST)

Evaluation of curative potentials of *F. platyphylla* leaf extract (FLE) was carried out as described by Ryley and Peters, [12]. Twenty five mice were selected and intraperitoneally injected with 1 x 10^7*P.berghei* infected erythrocytes. Seventy two hours after, the animals were divided into 5 groups of 5 animals each. Group 1 was administered normal saline (0.5ml/kg body weight), groups 2, 3 and 4 received 200, 400 and 600mg/kg body weight of FLE respectively while group 5 received 50 mg/kg body weight of artesunate. All administration of extracts, standard drug and normal saline were carried out daily through oral routes. Treatment continued until the seventh day and a thin film was prepared with blood collected from the tail of each mouse. The films were fixed with methanol, stained with Giemsa stain and parasitemia was ascertained by microscopic examination in five different fields.

EVALUATION OF PROPHYLACTIC ACTIVITY

The prophylactic activity of the extract was tested using the residual infection procedure described by Saidu *et al.,* [13]. Adult mice of both sexes were weighed and randomized into 5 groups of 5 mice each. Mice in group 1 were administered 0.5ml normal saline/kg body weight. Groups II, III and IV were administered 200, 400 and 600 mg FLE/kg body weight orally while group V received 50mg/kg body weight of artesunate orally daily for 5 days. On the fifth day, all the mice were inoculated with standard inoculum of 0.1 x 10^7*P. berghei berghei* NK 65 infected erythrocytes. Thin film of blood smears were made from each mouse 72 h after inoculation [14] and examined microscopically for the level of parasitaemia.

DETERMINATION OF PACKED CELL VOLUME (PCV)

The packed cell volume was evaluated using the method of Daice and Lewis [15]. Blood sample was collected into a heparinized capillary tube from the tip of the tail of each mouse and sealed with a plastacin. The tube was then centrifuge using a micro hematocrite centrifuge at 11,000rpm, for 5minutes. PCV was read using the micro haematocrite reader.

ETHICAL CLEARANCE

The ethical clearance for this study was approved by Federal University of Technology, Minna/Nigeria ethical review board (CUERB) in accordance with international standard on the care and use of experimental animals.

STATISTICAL ANALYSIS

A completely randomized design was used throughout this study and data was subjected to one-way analysis of variance and mean comparison with Duncan's Multiple Range Test (significance level of $P < 0.05$) using Statistic Package for Social Sciences (SPSS 22.0 for Windows: SPSS Inc., Chicago, IL, USA .

RESULT

PHYTOCHEMICAL SCREENING

The preliminary phytochemical test carried out on warm sample of petroleum ether leaf extracts of *F. platyphylla* is presented in Table 1. The analysis reviewed the presence of tannins, saponins, anthroquinones, flavonoids, and terpenoids while steriods, phenols, alkaloids and cardiac glycosides were not detected.

Table 1: Phytochemical Constituents of Petroleum Ether Leaf Extract of *F. platyphylla*

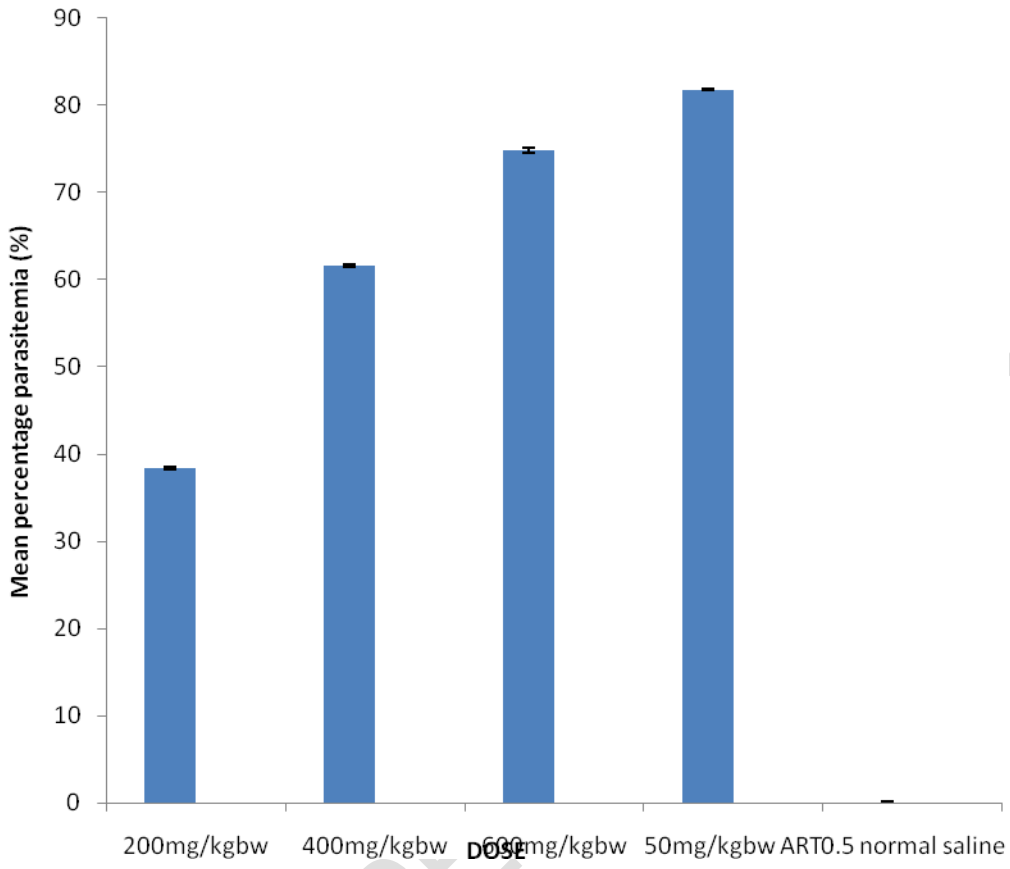
Bioactive Agent	Petroleum Ether Leaf Extracts
Tannins	+++

Saponin	++
Flavonoids	+++
Terpenoids	++
Steriods	+
Anthroquinones	+++
Phenol	–
Alkaloids	–
Cardiac glycosides	–

Trace(+); Moderate (++); High (+++); Absent (-)

RANE (CURATIVE TEST)

FLE produced significant dose-dependent decrease in parasite counts at ($p<0.05$). The mean percentage inhibition of parasitemia of the extract treated groups on day 7 were 38.4, 61.6 and 74.8% for groups administered with 200, 400, and 600mg/kg body weight of the extract respectively while that of the artesunate treated group was 81.8% (Fig 1)



135

136 **Fig 1: Percentage parasitemia in infected mice treated with FLE (curative test)**
137

138 The packed cell volume of infected mice administered with FLE before and after extract
139 administration is represented in Fig 2, from the result it can be deduced that there were
140 increase in the value obtained for PCV of mice in all treated groups but not in the negative
141 control which shows a reduction in the value of PCV throughout the study period.

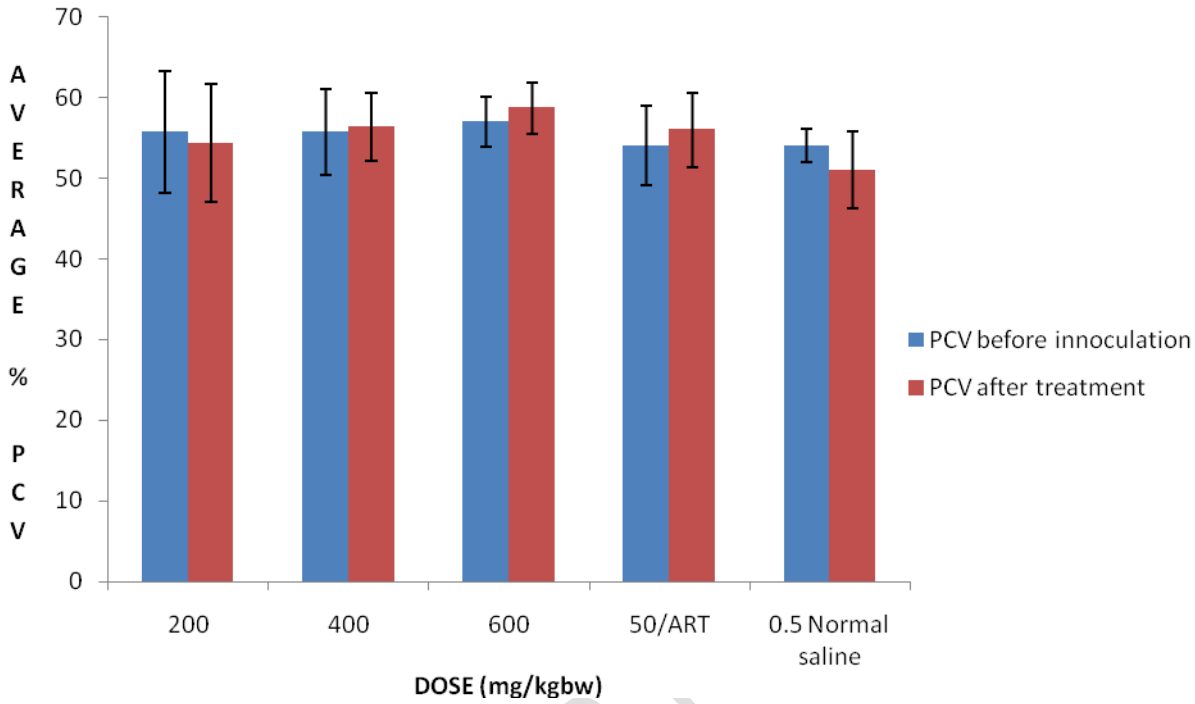


Fig 2: Effects of FLE on PCV in mice (Curative test).

The mean survival period in days were calculated to be 20.33± 0.67, 22.33±1.45, 27.00±2.08, 27.33±2.67 and 10.00±1.15 for 200, 400, 600mg/kg body weight of the plant extract and the control group (0.5ml normal Saline) respectively (Fig 3).

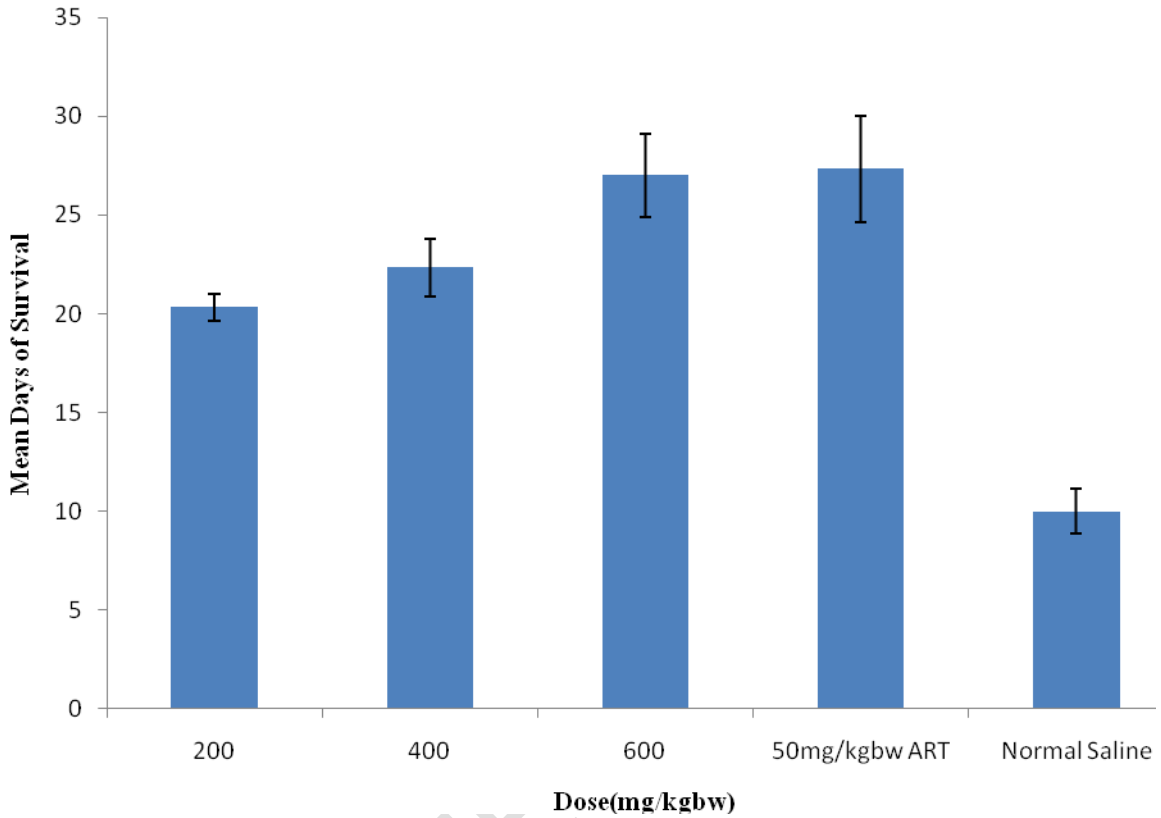
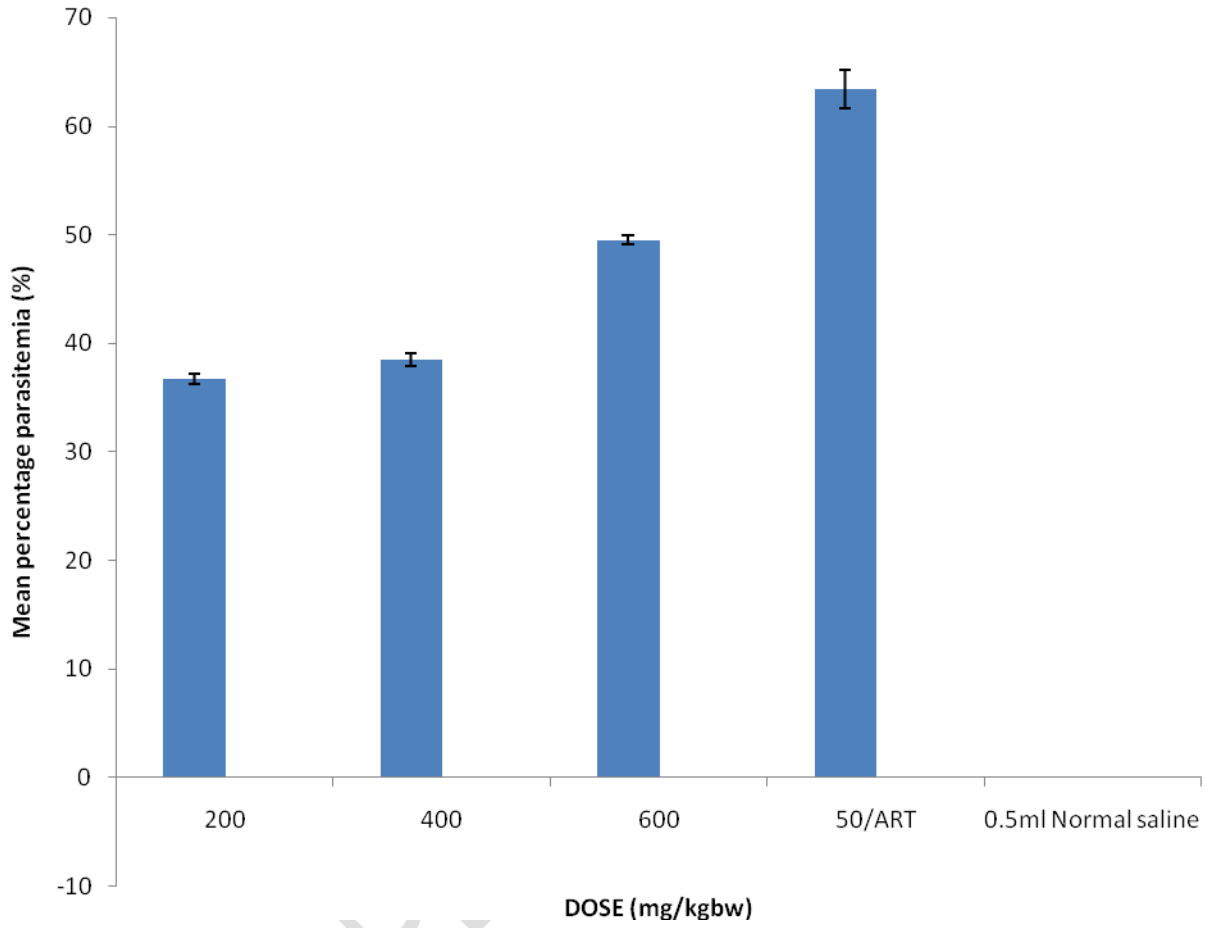


Fig 3: Mean days of survival of mice infected with *p. berghei* and treated with FLE for curative test.

PROPHYLACTIC TEST

FLE exhibited significant ($p<0.05$) dose dependent reduction in the level of parasiteamia; 36.7, 38.5, 49.5 and 63.4% at 200mg/kgbw, 400mg/kgbw, 600mg/kgbw and 50mg/kg body weight and artesunate treated groups respectively (Fig 4)



157

158 **Fig 4: Prophylactic effects of FLE against *P. bergei* infection in mice.**
159

160 There was a slight increase in PCV value for all the treated groups after extract
161 administration except for that of the negative control group in which no change in PCV value
162 was observed. However PCV value for all the treated groups drops 72hours after infection
163 with *p. berghei* (Fig 5).

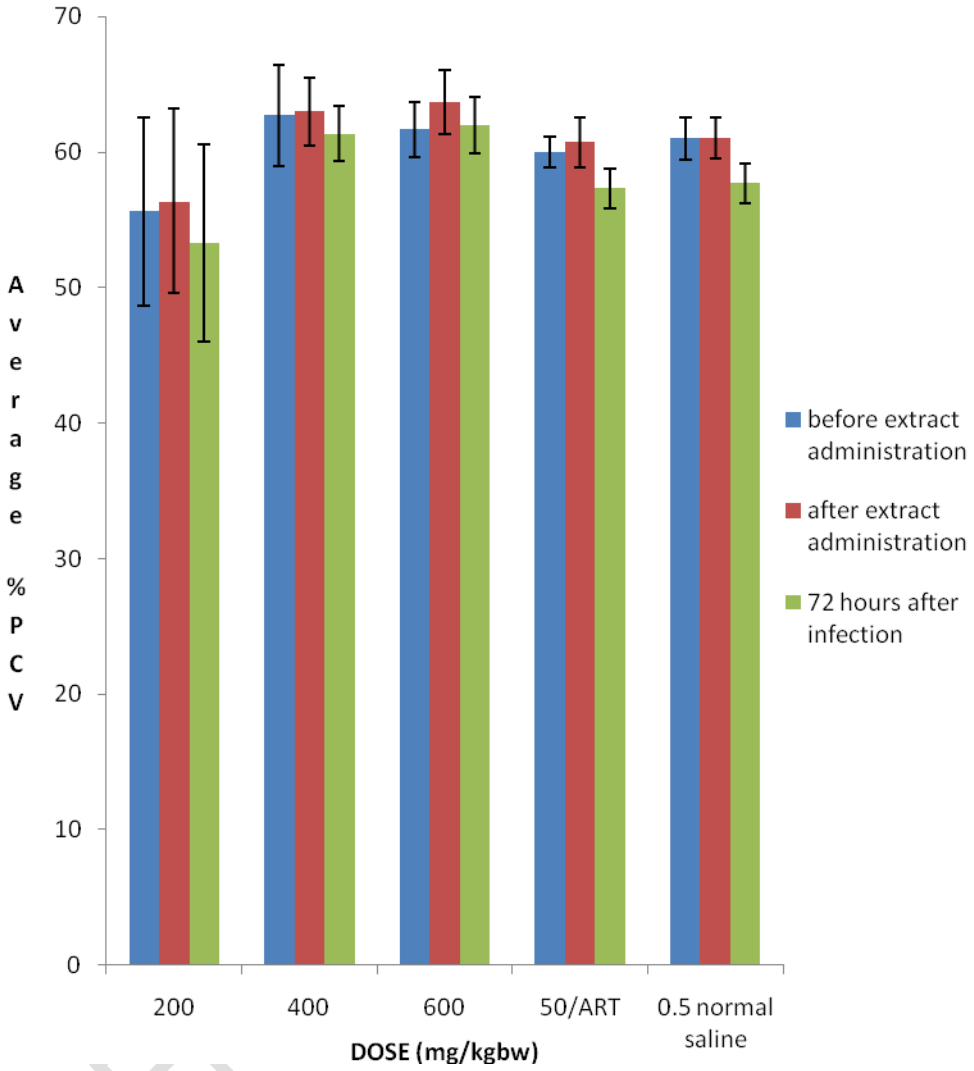


Fig 5: Effects of FLE on PCV in mice (Prophylactic test).

Fig 6 shows mean survival periods in days calculated to be 20.67±3.17, 21.0± 3.29, 25.33 ± 4.67, 26.67 ± 3.33 and 10.0 ± 1.0 for 200, 400, 600mg/kgbw of crude pet ether leaf extract of *F. platyphylla*, 50mg/kg body weight of artesunate and the control group (0.5ml of normal saline) respectively.

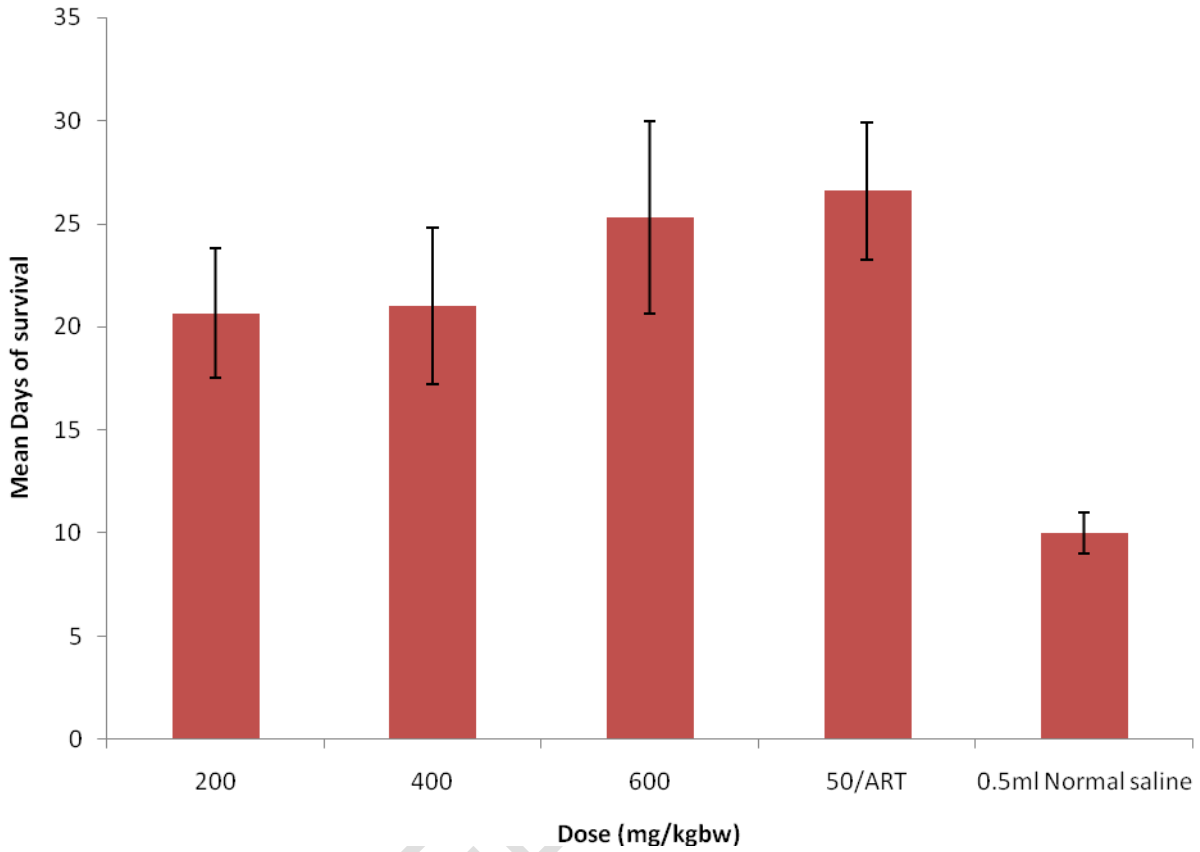


Fig 6: Mean days of survival of Mice infected with *P.berghei* and treated with FLE for prophylactic test

DISCUSSION

The present study was carried out to evaluate the antimalaria properties of *F. platyphylla* Del leave extract widely used in traditional treatment for malaria in some parts of Nigeria. Traditional remedies are common in regions where patients cannot afford to use chemically synthesized drugs. Poverty, traditional beliefs and moribund health centers have driven patients to use plants as the major source for treatment of various ailments [16].

The analytical result of qualitative phytochemical analysis of *F. platyphylla* showed the presence of tannins, saponins, flavonoids, terpenoids, steriods, anthroquinones and phenol, these findings agrees with the previous studies on the phytochemical constituents of

F.platyphylla [17, 18]. The observed antimalaria activity of FLE in this study may be attributed to the presence of saponin. These compounds have been previously shown to be responsible for the antimalaria activities in many plants. [19].

The determination of percentage inhibition of parasitemia has been noted to be a reliable parameter for assessment of antimalaria effect of a test compound [20]. With respect to the curative test, FLE exerted significantly suppressive effects in mice treated with 400mg/kgbw and 600mg/kgbw (Figure 3). This effect was however lower in group that received a lower dose while we observed a daily increase in parasitemia in the negative control group on day 7. The observed significant suppressive effects of FLE against *P. berghei* in mice is a confirmation of an earlier report in which stem bark ethanolic extracts of *F. platyphylla* significantly inhibited *Plasmodium berghei*, *invitro,* in mice [21]. Only one of the mice in the group administered 600mg/kgbw of petroleum ether leaf extract survived up to 28 days, when compared with the experimental animal in the artesunate-treated group, where two (2) of the animals survived beyond 28 days.

PCV is a widely known index of anemia [22], FLE was able to prevent a drastic reduction in PCV in infected mice when compare to infected untreated experimental control, thus, showing its efficacy in ameliorating malaria-induced anemia. This was consistent with the marked decrease in parasite load observed in the cause of infection in the groups of mice treated with the 400mg/kg body weight and 600mg/kg body weight doses of FLE. The increase in PCV of extract-treated groups, as well as artesunate-treated groups, may be as a result of clearance of the parasite from circulation thereby enabling the cells to gradually divide and replenish the blood [23].

In the prophylactic study, FLE significantly at ($p<0.05$) exerted a dose dependent reduction in the parasitemia level in the extract-treated groups while the standard drug artesunate has the highest chemo-suppressive effect. Although the result from the antimalaria study of the crude petroleum ether extract of *F. platyphylla* suggest that the extract has more curative effect, than prophylactic effect as evident from the percentage prophylaxis (Figure 4). This

low activity may be due to rapid hepatic clearance of the active component of the plant extract and so parasite clearance may not be completely cleared from the blood stream.

The level of packed cell volume of the mice in FLE-treated groups and that of the untreated group drastically reduced 72 hours after inoculation of parasite, which may be as a result of short duration of action of the extract occasioned by rapid metabolism. As a result, *plasmodium* parasites which are usually localized in cell lyse the red blood cell and thus the percentage packed cell volume is affected. The survival rate of mice infected with *plasmodium berghei* and treated with crude pet ether leaf extract compares favourably with group treated with standard artesunate.

CONCLUSION

The result shows that *F. platyphylla* leaf extracts exhibited some antimalaria properties as claimed by local herbal traditionalist, thus justifying their long use in local malaria treatment. Hence, the plant could be exploited for safe, effective and affordable antimalaria regimen.

COMPETING INTEREST

There are no competing interests among authors.

CONSENT

It is not applicable

ETHICAL CLEARANCE

The ethical clearance for this study was approved by Federal University of Technology, Minna/Nigeria ethical review board (CUERB) in accordance with international standard on the care and use of experimental animals.

REFERENCES

1 World Health Organisation (WHO). Geneva, world malaria report, Global Malaria programme. 2011.

2 World Health Organization, World Malaria Report. Geneva, Global Plan for Insecticide Resistance Management in Malaria Vectors (GPIRM). 2011; 13.

3 Lamar, J., Martschinske, R., Tetreault, G., and Doud, C. Navy Medical Department Pocket Guide to Malaria Prevention and Control. 2007 1-112.

4 Nadembega, P, Boussim, JI, Nikiema, JB, Poli, F. and Antognoni, F. "Medicinal plants in baskoure, kourittenga province, Burkina Faso: an ethnobotanical study," *Journal of Ethnopharmacology*, 2011; vol. 133, no. 2, pp. 378–395.

5 Chindo, B., Joseph A., and Amos, A. Anticonvulsant properties of Saponins from *Ficus platyphylla* Stem Bark. Brain Research Bulletin 2009; 78 Issues 6: 276-282.

6 Chindo, BA., Amos, S., Odutola, AA., Vongtau, HO., Abbah, J., Wambebe, C., & Gamaniel, KS. Central nervous system activity of the methanol extract of *Ficus platyphylla* stem bark. *Journal of Ethnopharmacological,* 2003. 85:131–137.

7 Jigam, AA, Usman, TA, Halima, AM and Tijani, FO. Efficacy of thonningia sanguine Vahl root extract against plasmodium chabaudi, inflammation and nociception in mice. *Journal of applied pharmaceutical science*, 2012; 02 (01); 47-51.

8 Canadian Council of Animal Care (CCAC) Guidelines and Protocol Review. 1997

9 Kabiru AY, Okolie NL, Muhammad HL, and Ogbadoyi EO. Preliminary studies on the antiplasmodial potential of aqueous and methanol extracts of *Eucalyptus camaldulensis*. *Asian Pacific Journal of Tropical Disease*, 2012; S809-S814.

10 Ogbadoyi, EO, Akinsunbo OA, Adama, TZ, Okogun, JI. In vivo trypanocidal activity of *Annona senegalensis* Pers. leaf extract against *Trypanosoma brucei brucei*. *Journal of Ethnopharmacology,* 2007;112: 85-89.

11 Odebiyi, OO, and Sofowora, ES. Antimicrobial alkaloids from a Nigerian chewing stick (Fagaraz anthoxyloides). *Planta Medicals,* 1979; 36, 204–207.

12 Ryley, JF, Peters, W. The antimalarial activity of some quinone esters. Annals of *Tropical Medicine and Parasitology* 1970; 84, 209–222.

13 Saidu, K, Onah, J, Orisadipe, A, Olusola, A, Wambebe, C, Gamaniel, K. Antiplasmodial, analgesic, and anti-inflammatory activities of the aqueous extract of the stem bark of *Erythrina senegalensis*. *Journal of Ethnopharmacology* 2000; 71, 275–280.

14 White N, Nosten F, Bjorkman A, Marsh K, Snow RW. *The Lancet,* 2004; 363: 1160.

15 Daice, JV., & Lewis, SM. Practical Haematology, 5th Edition, Churchill-living stone, Edinburgh. 1975.

16 Tshibangu, JN, Chifundera, K, Kaminsky, R, Wright, AD, Konig, GM. Screening of African medicinal plants for antimalarial inhibitory activity. *Journal of Ethnopharmacol*ogy 2002; 80: 25-35.

17 Ugwah- Oguejiofor, CJ, Bello, SO, Etuk, EU, Igbokwe, V U, Ugwah, OM and Okolo RU. Preliminary toxicity and phytochemical studies of the aqueous extract of *Ficus* platyphyllain female albino rats*. International research journal of pharmacy and pharmacology,* 2011; Vol 1 (5) pp. 086- 092.

18 Olugbenga AM, Musa, AE, and Oluwatoyin, AH. Toxicological activity of crude saponin extract of *ficus platyphylla,Asian journal of pharmaceutical and clinical research*, 2012; Vol 5(1) pp 30-33.

19 Okokon, J, Ofodum, KC, Ajibesin, KK, Danlandi, B, and Gamaneil. KS. Pharmacological screening and evaluation of antiplasmodial activity of *Croton zambesicus* against *P. berghei* infection in mice. *Indian Journal of Pharmacology, 2005;* 37:243-246.

20 Salawu, OA, Tijani, AY, Babayi, H, Nwaeze AC, Anagbogu, RA, and Agbakwuru, VA. Anti- malaria activity of ethanolic stem bark extract of *FaidherbiaAlbida (Del)* a. Chev (Mimosoidae) in mice; *Archives of applied science research*, 2010; 2 (5): 261-268.

21 Shittu, I., Emmanuel, A., Nok, AJ. Antimalaria effect of the ethanolic stem bark extracts of *Ficus platyphylla* del. *Journal of parasitological research,* 2011 10(5), 1155- 1160.

22 Esievo, KAN, Saror, DL, Ilemobade, AA, and Hallaway, AM. Variation in erythrocytes surface and free serum sialic acids concentration during experimental trypanosome vivax infection in cattle, *Research in vet sci,* 1982; 32, (1) 1-5.

23 Kabiru, AY, Abdulkadir, A, Gbodi, AT, Bello, UM, Makun HA, Amah, DJ. and Ogbadoyi, EO. Evaluation of haematological changes in plasmodium- berghei-infected mice administered with aqueous extract of phyllantus amarus, *Pakistan journal of biological science*, 2013; Vol 16 (11): 510- 516.